Fibrosis: from mechanisms to medicines

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Fibrosis can affect any organ and is responsible for up to 45% of all deaths in the industrialized world. It has long been thought to be relentlessly progressive and irreversible, but both preclinical models and clinical trials in various organ systems have shown that fibrosis is a highly dynamic process. This has clear implications for therapeutic interventions that are designed to capitalize on this inherent plasticity. However, despite substantial progress in our understanding of the pathobiology of fibrosis, a translational gap remains between the identification of putative antifibrotic targets and conversion of this knowledge into effective treatments in humans. Here we discuss the transformative experimental strategies that are being leveraged to dissect the key cellular and molecular mechanisms that regulate fibrosis, and the translational approaches that are enabling the emergence of precision medicine-based therapies for patients with fibrosis.

Fibrosis is not a disease but rather an outcome of the tissue repair response that becomes dysregulated following many types of tissue injury, most notably during chronic inflammatory disorders. The formation of fibrotic tissue, which is defined by the excessive accumulation of extracellular matrix (ECM) components such as collagen and fibronectin, is in fact a normal and important phase of tissue repair in all organs. When tissues are injured, local tissue fibroblasts become activated and increase their contractility, secretion of inflammatory mediators, and synthesis of ECM components; together, these changes initiate the wound healing response. When damage is minor or non-repetitive, wound healing is efficient, resulting in only a transient increase in the deposition of ECM components and facilitating the restoration of functional tissue architecture. However, when the injury is repetitive or severe, ECM components continue to accumulate, which can lead to disruption of tissue architecture, organ dysfunction and ultimately organ failure. Notably, studies of tissue repair in embryonic and fetal mice and human fetal surgery have shown that before the onset of the wound inflammatory response, immature tissues are capable of scarless healing, suggesting that inflammation might be a cause of fibrosis[1]. However, in adult mammalian tissue, ageing, the response to invading microorganisms, and the changing character of the inflammatory response over time influence whether wound healing responses lead to progressive fibrosis or end in efficient repair. Genetics is also important; specific mutations and rare variants that are associated with fibrosis have revealed antifibrotic targets and core pathways that might be druggable. The genes involved include *MUC5B* in pulmonary fibrosis[2], *MYH7* in cardiac fibrosis[3], and *DMD* in Duchenne muscular dystrophy-associated skeletal muscle fibrosis[4]. Such genetic alterations suggest the involvement of non-fibroblast cell types that act upstream of mesenchymal cell activation. These findings emphasize the importance of multicellular interactions in the pathogenesis of fibrosis. In this review, we provide an update on recent research into the mechanisms of fibrosis and discuss how this information is enabling the development of antifibrotic treatments.

Single-cell genomics of fibrosis

Single-cell multi-omics approaches are transforming our understanding of disease pathogenesis across medicine, making it possible to study cell populations in health and disease at unprecedented resolution. This 'resolution revolution' allows the powerful unbiased exploration of cell states and types at single-cell level, resulting in unexpected insights into tissue biology and disease mechanisms (Fig. 1).

These cutting-edge single-cell approaches have already been avidly adopted by the fibrosis research community to deepen our understanding of the complex, multicellular interplay that drives lung fibrosis[5]. Mesenchymal cells are the key source of pathological ECM deposition during lung fibrosis, which ultimately leads to architectural disruption and reduced lung function. Spatial transcriptional maps of the mouse lung mesenchyme have been generated by combining single-cell RNA sequencing (scRNA-seq) and signalling lineage reporters[6]. Each mesenchymal lineage demonstrated a distinct spatial address and transcriptome, in turn conferring distinct fibrotic niche regulatory functions across these mesenchymal subpopulations. Examples include mesenchymal cells in the alveolar niche that express *Pdgfra* and respond to Wnt signalling, and are critical for the growth and self-renewal of alveolar epithelial cells. By contrast, *Axin2*$^{+}$ myofibrogenic progenitor cells preferentially generated pathologically deleterious myofibroblasts after lung injury[6]. Further studies using the mouse model of bleomycin-induced lung injury have also identified lung mesenchymal cell heterogeneity in both healthy and fibrotic mouse lungs[7–9].

The analysis of more than 70,000 cells of multiple lineages from eight lung explants from patients with pulmonary fibrosis (of varying aetiologies) and eight lung samples from healthy donors[10] identified a distinct population of pro-fibrotic alveolar macrophages, which had previously been characterized in mice[10,11], in the samples from patients with fibrosis. This study and others[10,12] have suggested that alveolar type 2 (AT2) cells, which secrete pulmonary surfactant and serve as alveolar stem cells, have a pathological role. These results identified

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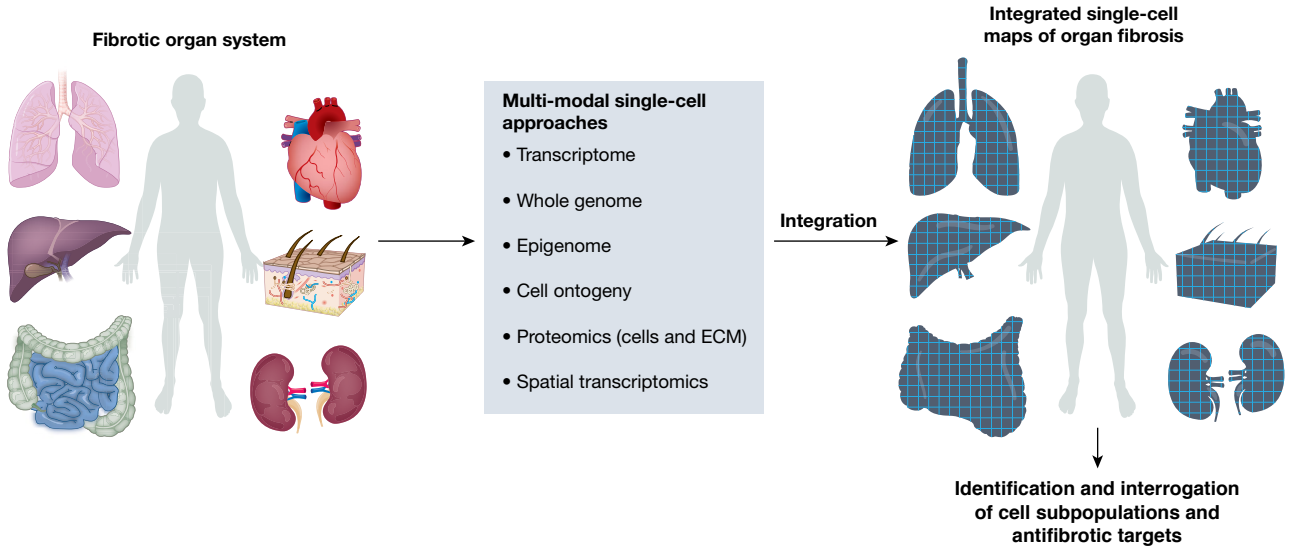

Fig. 1 | Deconvolving fibrosis using multi-modal single-cell approaches. Cutting-edge single-cell approaches are transforming our understanding of the complex cellular and molecular mechanisms that regulate fibrosis and making it possible to assess the transcriptome, genome, epigenome and proteome at a single-cell level, in addition to spatial profiling. Furthermore, combined readouts from the same single cell are now possible (for example, the simultaneous profiling of transcriptome and chromatin accessibilty), and integration of these multi-modal single-cell omics readouts has allowed ever more powerful, comprehensive assessments of cell state, ontogeny, phenotype and function during human fibrotic disease. The new biological insights gained from these integrated approaches should enable the identification of novel and tractable therapeutic targets to treat patients with a broad range of fibrotic diseases.

a distinct population of AT2 cells in fibrotic lungs[13] and established a direct mechanistic link between elevated TGFβ signalling induced by mechanical tension caused by impaired alveolar regeneration, and progressive lung fibrosis. Decreasing the mechanical tension on alveoli could be a therapeutic approach for treating progressive lung fibrosis[13]. A study that profiled 312,928 lung cells from 32 patients with idiopathic pulmonary fibrosis (IPF), 18 patients with chronic obstructive pulmonary disease (COPD) and 29 healthy control individuals identified a population of aberrant basaloid epithelial cells located at the edge of myofibroblast foci that were enriched in the lungs of patients with IPF[14]. Within the vascular endothelial cell compartment, samples from patients with IPF contained an expanded cell population that was transcriptomically identical to vascular endothelial cells that are normally restricted to the bronchial circulation. Furthermore, diffusion map and pseudotemporal trajectory analyses (computational techniques used in single-cell transcriptomics to determine the pattern of a dynamic process experienced by cells, and then to arrange cells according to their progression through the process) made it possible to infer the origins of activated myofibroblasts in IPF[14].

scRNA-seq has also been used to comprehensively profile the cellular and molecular landscape in liver homeostasis and regeneration[15–18]. Since their discovery as major collagen-producing cells in the liver[19], hepatic stellate cells (HSCs) have been considered a homogenous population, with equal potential to transition to the activated, myofibroblast phenotype. However, scRNA-seq has shown that mouse HSCs can be divided into functional zones, allowing high-resolution identification of the critical pathogenic collagen-producing cells in livers with centrilobular injury[20]. Pseudotemporal trajectory and RNA velocity, another computational approach that predicts the future state of individual cells on a timescale, demonstrated that central vein-associated HSCs are the dominant source of pathogenic collagen-producing cells following centrilobular liver injury[20]. Furthermore, the use of scRNA-seq to interrogate retinol-positive myofibroblasts isolated from fibrotic mouse livers has also shown that liver myofibroblasts are heterogeneous and functionally diverse[21].

The profiling of more than 100,000 human liver cells yielded molecular definitions for non-parenchymal cell types that are found in the healthy and cirrhotic human liver and identified a scar-associated subpopulation of macrophages that express triggering receptor expressed on a myeloid cell-2 (TREM2) and CD9, which expands in liver fibrosis, differentiates from circulating monocytes and is pro-fibrogenic[22]. Endothelial cell subpopulations that express disease-associated atypical chemokine receptor-1 (ACKR1) and plasmalemma vesicle-associated protein (PLVAP), which are topographically restricted to the fibrotic niche and enhance the transmigration of leucocytes, were also defined. Multi-lineage modelling[23,24] of ligand–receptor interactions among the scar-associated macrophages, endothelial cells and PDGFRα$^+$ collagen-producing mesenchymal cells revealed that several pro-fibrogenic pathways were active in scars, including TNF receptor superfamily (TNFRSF) 12A, PDGFR and NOTCH signalling, which provides a conceptual framework for the discovery of rational therapeutic targets in cirrhotic livers[22]. Macrophages associated with liver injury in mice show substantial overlap of marker genes with human scar-associated macrophages, including the expression of TREM2 and CD9 in both species[22]. Unbiased cross-species mapping of scRNA-seq data using canonical correlation analysis (CCA)[25] confirmed that mouse and human scar-associated macrophages represent corollary populations. This shows that scRNA-seq approaches can be useful for defining 'core' fibrotic injury-induced populations and therapeutic targets across species, thereby increasing precision in the interrogation of putative targets across the translational pipeline, from preclinical rodent models to human liver primary cell or organoid-based systems. Although the treatment of liver fibrosis has met with disappointing failures over the past few years[26], including late-stage readouts for Elafibranor, a dual PPARα/δ agonist (NCT02704403), and Selonsertib, an ASK1 inhibitor (NCT03053050), clinical studies of Ocaliva (obetacholic acid; NCT03836937), Cenicriviroc (CCR2/5 dual antagonist; NCT03028740), Aramchol (a fatty acid bile acid conjugate; NCT04104321), MGL-3196 (liver-directed, thyroid hormone receptor (THR) β-selective agonist; NCT03900429), granulocyte

colony-stimulating factor (GCSF; NCT03911037), and a combination study of diacylglycerol *O*-acyltransferase-2 (DGAT2) and acetyl-CoA carboxylase (ACC) inhibitors (NCT04321031) have shown promise and are undergoing further testing. In addition, omics and genetic analyses are beginning to better inform patient selection and stratification, and it is hoped that this will facilitate improved outcomes.

In the gastrointestinal tract[27], a single-cell census of the human colonic mesenchyme revealed four subsets of fibroblasts in addition to pericytes and myofibroblasts, and identified a fibroblast subpopulation proximal to the colonic crypt niche that expressed *SOX6*, *F3* (also known as *CD142*), and WNT genes, which are essential for colonic epithelial stem cell function. In colitis, this niche became dysregulated; an activated mesenchymal population emerged that expressed *TNFSF14*, genes associated with fibroblastic reticular cells, *IL33*, and lysyl oxidase (*LOX*). These cells led to impaired epithelial proliferation and maturation, thus illustrating how the colonic mesenchyme remodels to drive inflammation and barrier dysfunction in inflammatory bowel disease (IBD)[27].

In the context of arthritis, deletion of fibroblasts expressing fibroblast activation protein-α (FAPα) suppressed inflammation and bone erosion in mouse models of resolving and persistent arthritis. Single-cell RNA-seq identified two anatomically distinct fibroblast subsets within the FAPα$^+$ population: FAPα$^+$ thymus cell antigen (THY1)$^+$ immune 'effector' fibroblasts in the synovial sub-lining, and FAPα$^+$THY1$^-$ 'destructive' fibroblasts restricted to the synovial lining layer. Adoptive transfer of FAPα$^+$THY1$^-$ fibroblasts into the joint selectively mediated bone and cartilage damage with little effect on inflammation, whereas transfer of FAPα$^+$ THY1$^+$ fibroblasts resulted in a more severe and persistent inflammatory arthritis, with minimal effect on bone and cartilage. The discovery of these anatomically discrete, functionally distinct subsets of fibroblasts with non-overlapping functions has important implications for the rational design of therapies aimed at precisely modulating inflammation, fibrosis and tissue repair[28].

Single-cell RNA-seq studies are also beginning to shed new light on the mechanisms that regulate kidney injury and fibrosis[29–31]. For example, recent work using single-nucleus RNA-seq in a mouse model of acute kidney injury identified a distinct pro-inflammatory and pro-fibrotic proximal tubule cell state that fails to repair. Deconvolution of bulk RNA-seq data sets showed that this failed-repair proximal tubule cell (FR-PTC) state can be detected in other models of kidney injury, and that it increases during ageing in rat kidney and over time in human kidney allografts[29]. Furthermore, a recent study has used scRNA-seq to profile the transcriptomes of proximal and non-proximal tubule cells in healthy and fibrotic human kidneys, enabling mapping of all matrix-producing cells at high resolution. This revealed distinct subpopulations of pericytes and fibroblasts as the major cellular sources of scar-forming myofibroblasts during human kidney fibrosis. Genetic fate-tracing, time-course scRNA-seq and assay for transposase-accessible chromatin (ATAC)–seq experiments in mice, and spatial transcriptomics in human kidney fibrosis, were then used to functionally interrogate these findings, identifying *Nkd2* as a myofibroblast-specific target in human kidney fibrosis[30]. Recent studies in which scRNA-seq has been used to investigate mechanisms of fibrosis in various organ systems are summarized in Supplementary Table 1.

Fibroblast heterogeneity and plasticity

Functional fibroblast heterogeneity

Increasingly sophisticated experimental approaches have revealed substantial diversity and functional heterogeneity within the fibroblast population during organ fibrosis[27,28,32–35]. A combination of fate mapping and live imaging showed that a specialized subset of fibroblasts, fascia fibroblasts, rise to the surface of the skin after wounding[36]. These fascia fibroblasts gather their surrounding ECM (including blood vessels, macrophages and peripheral nerves) to form the provisional matrix, and ablation of these fibroblasts inhibits matrix homing into wounds and leads to defective scars. Notably, the placement of an impermeable film beneath the skin (preventing upward migration of fascia fibroblasts) led to chronic open wounds. Thus, the fascia contains a specialized prefabricated kit of sentry fibroblasts, which are embedded within a movable sealant. Whether similar fibroblast subpopulations exist in other organs and use analogous mechanisms to promote wound healing remains to be determined. There is also substantial functional diversity among myofibroblasts during skin injury and ageing[37]. Lineage tracing and flow cytometry identified distinct subsets of wound bed myofibroblasts, including CD26-expressing adipocyte precursors and a CD29^{high} subpopulation. Wound beds in aged mice or in bleomycin-induced fibrotic mouse skin showed a decrease in adipocyte precursors and an increase in CD29^{high} cells compared to young healthy mice, suggesting that the fibrotic microenvironment alters the composition and function of myofibroblasts. Senesecence, a state in which cells cease to divide, also influences the fate and function of fibroblasts. However, whether fibroblast senescence plays a benefical or detrimental role in inflammation, tissue repair or fibrosis remains unclear, and its effects may vary in different tissues and types of disease[38–40]. Some studies have suggested that senescent fibroblasts become resistant to apoptosis, thereby sustaining inflammation and fibrosis through their production of inflammatory cytokines, immune modulators, growth factors and proteases. Consequently, senotherapeutic and senolytic drugs have emerged as potential new treatments for fibrosis and related ageing-associated diseases[39,41].

Recent studies have implicated a range of mesenchymal progenitor cells (MPCs) in the initiation and propagation of fibrosis[42,43]. In particular, two studies, focusing on populations of MPCs expressing HIC1, PDGFRα and LY6A in the heart and skeletal muscle, have demonstrated a hierarchy of MPCs, diversity in the pathophysiological roles of their progeny, and how fate determination of MPCs is context dependent[44,45]. Conditional genetic inactivation of *Hic1* in mice led to activation and expansion of MPCs in both heart and skeletal muscle, demonstrating that HIC1 is required for maintaining MPC quiescence. In the heart, HIC1 deficiency (in PDGFRα-expressing cells) led to activation of MPCs and accumulation of cardiac fibroadipogenic progenitor cells, with epicardial thickening, interstitial fibrosis and fibrofatty depositions resulting in pathological features that are pathognomonic of arrhythmogenic cardiomyopathy[45]. However, although inactivation of *Hic1* in skeletal muscle also resulted in a marked expansion of PDGFRα$^+$LY6A$^+$ cells during homeostasis, this did not increase skeletal muscle fibrosis and had no substantial effect on skeletal muscle regeneration[44], highlighting the diverse pathophysiological roles of these cells in different organs.

Fibrosis, secondary to age-associated chronic low-grade inflammation, is increasingly recognized as an important cause of morbidity and mortality. Increasing age is a driver of functional heterogeneity in fibroblasts, with 'old' fibroblasts demonstrating variability in their ability to reprogram and heal wounds[46]. Old mice showed varying wound healing rates in vivo, and scRNA-seq identified distinct subpopulations of fibroblasts with differing cytokine expression profiles in the wounds of old mice with slow versus fast healing rates. This increased variability in wound healing with increasing age may reflect distinct stochastic ageing trajectories between individuals, which will need to be considered when designing personalized antifibrotic therapies for the elderly population[46].

Fibroblast targeting and reprogramming

Fibroblasts, as well as displaying substantial functional heterogeneity, are capable of remarkable plasticity and phenotype switching during the progression and regression of fibrosis[43,47,48]. For example, the transcription factor PU.1 (also known as SPI1) has a major role in fibroblast polarization and fibrogenesis[49]. PU.1 both polarizes resting fibroblasts and repolarizes ECM-degrading inflammatory fibroblasts to an ECM-producing fibrotic phenotype. Furthermore, inactivation

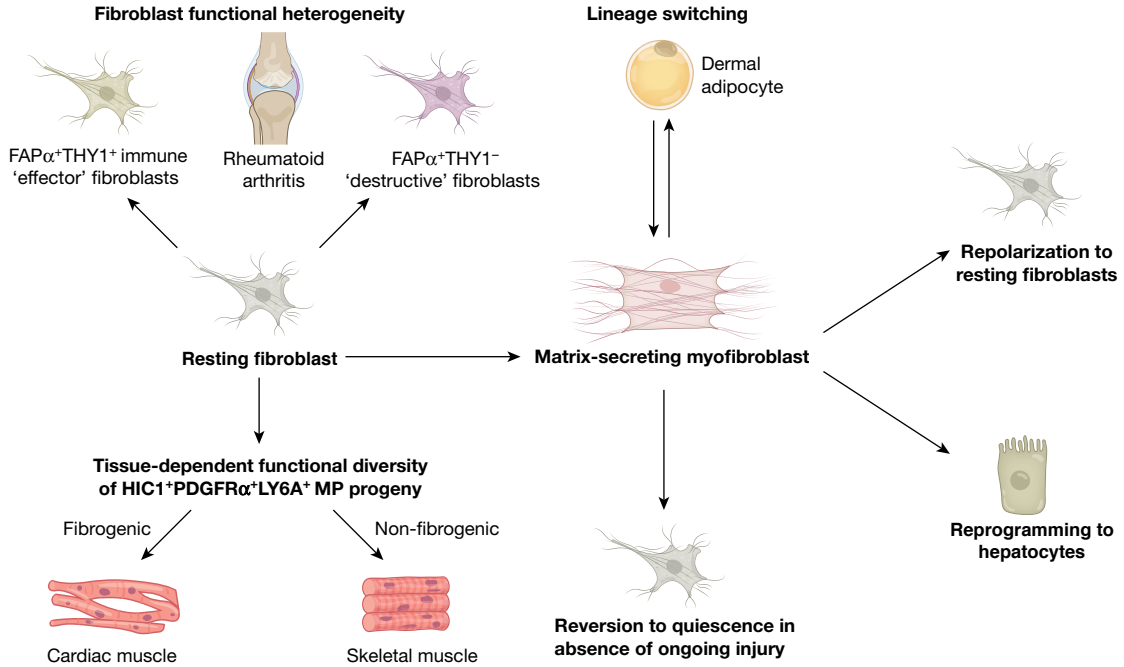

Fig. 2 | Functional heterogeneity and plasticity of fibroblasts. Fibroblast populations show substantial functional heterogeneity and plasticity during fibrosis. In the context of arthritis, scRNA-seq combined with adoptive transfer experiments has been used to identify two anatomically distinct fibroblast subsets within the FAPα$^+$ population: FAPα$^+$ thymus cell antigen (THY1)$^+$ immune 'effector' fibroblasts located in the synovial sub-lining, and FAPα$^+$THY1$^-$ 'destructive' fibroblasts that are restricted to the synovial lining layer. Studies of MPC populations (HIC1$^+$PDGFRα$^+$LY6A$^+$) in heart and skeletal muscle have demonstrated a hierarchy of MPCs, diversity in the pathophysiological roles of their progeny, and how fate determination of MPCs is tissue-dependent. Fibroblasts are also capable of remarkable plasticity and phenotype switching during the progression and regression of fibrosis. Myofibroblasts can revert to a quiescent state in the absence of ongoing injury, or undergo full lineage switching with adipocytes (as observed during cutaneous wound healing in mice). Furthermore, genetic and pharmacological inactivation of the transcription factor PU.1 can reprogram fibrotic fibroblasts into resting fibroblasts, resulting in the regression of fibrosis in several organs. Finally, viral vector-mediated expression of specific transcription factors in myofibroblasts in the liver has been used to reprogram myofibroblasts into hepatocyte-like cells in fibrotic mouse livers, thereby reducing liver fibrosis and increasing liver function.

of PU.1 enabled fibrotic fibroblasts to be reprogrammed into resting fibroblasts, leading to the regression of fibrosis[49]. There is also remarkable inter-lineage plasticity between myofibroblasts and other cell types during fibrosis. During cutaneous wound healing in mice, adipocytes were regenerated from myofibroblasts. This reprogramming required neogenic hair follicles, which triggered bone morphogenetic protein (BMP) signalling and the activation of adipocyte transcription factors that are expressed during development[50]. Furthermore, adipocytes were generated from human keloid fibroblasts when treated with BMP in vitro, or when placed with human hair follicles[50]. Viral vector-mediated expression of specific transcription factors in liver myofibroblasts has been used to reprogram myofibroblasts into hepatocyte-like cells in fibrotic mouse livers, thereby reducing liver fibrosis and increasing liver function[51,52]. The ability to selectively target scar-producing myofibroblasts during fibrosis and to reprogram these cells into other lineages that support organ function opens up exciting new avenues for antifibrotic and pro-regenerative therapies.

Together, these studies highlight the profound diversity, functional heterogeneity and plasticity of fibroblasts during fibrosis, both within and between organs (Fig. 2). More precise delineation of fibroblast heterogeneity and phenotype in different disease settings should facilitate the design of rational, highly targeted antifibrotic therapies, ultimately allowing the specific inhibition, ablation, or reprogramming of pathological fibroblast subpopulations while preserving essential, homeostatic fibroblast function. For example, adoptive transfer into mice of CD8$^+$ T cells expressing a chimaeric antigen receptor against FAP led to the selective ablation of pathogenic fibroblasts and a substantial decrease in cardiac fibrosis following injury[53]. Boosting natural killer (NK) cell responses by blocking the NK cell receptor NKG2A has also been proposed as a mechanism to eliminate senescent fibroblasts in skin[54]. Finally, one of the first trials to explore fibroblast cellular therapy was a phase 2 study that used allogeneic human dermal fibroblasts to remodel contracted scars (NCT01564407; Supplementary Table 2).

The dynamic matrisome

During wound healing, the ECM is critical for mechanically stabilizing injured tissue, immobilizing growth factors and acting as a scaffold for the migration of fibroblasts, immune cells and endothelial cells into areas of tissue injury and repair[55]. As such, the ECM is increasingly appreciated as a highly dynamic entity that can influence the progression and resolution of fibrosis via a range of mechanisms. The fibrotic matrix directly promotes myofibroblast activation through mechanotransduction pathways, which convert mechanical signals (changes in stiffness) into biochemical responses. For example, in a pig model of incisional skin wounding, mechanical loading of wounds upregulated the expression of genes associated with fibrosis, whereas mechanical offloading of these wounds reversed this effect[56]. The increased mechanical strain within the stiffened matrix also provides a direct mechanism for the conversion of latent TGFβ1 into its active form[57]. Finally, through the actions of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), myofibroblasts continuously regulate matrix deposition and turnover[58,59]. Advances in mass spectrometry, proteomics, and spatial proteomics[60] should greatly accelerate our understanding of how changes within the matrisome itself maintain tissue fibrogenesis independently of inflammatory signals.

Metabolic regulation of mesenchymal cells

It is now apparent that cells involved in the progression and resolution of fibrosis are metabolically 'reprogrammed' to perform distinct

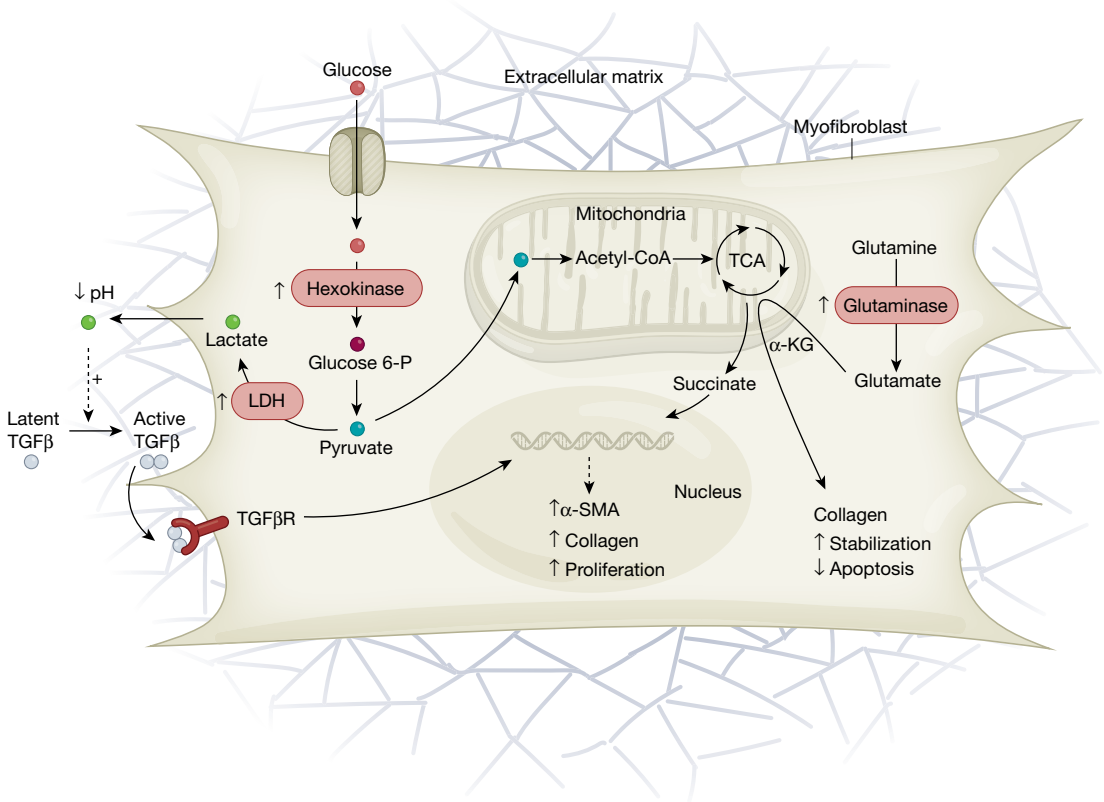

Fig. 3 | Metabolomic reprogramming of activated fibroblasts. Pro-fibrotic fibroblasts increase glycolysis via hexokinase, which leads to increased pyruvate and lactate. Lactate decreases extracellular pH and activates latent TGFβ1. Pyruvate also feeds the tricarboxylic acid (TCA) cycle after conversion into acetyl-CoA and thereby increases succinate levels. Both mechanisms lead to an increase in α-SMA, collagen production and cell proliferation. Glutaminase activity is increased, and this converts glutamate to glutamine, which is converted into α-ketoglutarate (α-KG) via the TCA cycle; this decreases apoptosis and enhances collagen stabilization. LDH, lactate hydrogenase; P, phosphate.

functions during tissue repair. The effects of metabolism have been explored extensively in the context of non-alcoholic steatohepatitis (NASH)-driven fibrosis, in which dysregulated hepatic lipid metabolism serves as a key driver of liver injury and cirrhosis[61]. As discussed earlier, fibroblasts are the key source of ECM deposition during fibrosis. Hence, metabolic alterations of local tissue mesenchymal cells may offer future therapeutic avenues that include the major carbohydrate, amino acid and lipid metabolism pathways (Fig. 3).

Following tissue injury, mesenchymal cells undergo profound metabolic changes to facilitate energy-consuming cellular functions such as proliferation and protein synthesis[62]. In fibroblasts, aerobic glycolysis is increased by upregulating rate-limiting glycolytic enzymes[62]. As well as providing a rapid energy-generating mechanism compared to oxidative phosphorylation, glycolysis produces by-products such as lactate that regulate fibrosis. A reduction in extracellular pH, combined with an increase in lactic acid, promotes myofibroblast differentiation by activating TGFβ1, and lactate itself may serve as an additional source of energy for mesenchymal cells[63,64]. Activation of fibroblasts increases key glycolytic enzymes, such as hexokinase 2 and lactate dehydrogenase[65], which in turn increase cell proliferation[65] and collagen synthesis[66]. During enhanced glycolysis, increased amounts of pyruvate are converted into acetyl-CoA in the mitochondrial matrix[65] before entering the citric acid cycle. This yields intermediate metabolites, such as succinate, which promote fibrosis[67]. Disregulated glycolysis has been implicated in experimental models of lung, liver and kidney fibrosis, and inhibition of glycolysis reduces ECM accumulation[68–70]. During fibrogenesis, mesenchymal cells also exploit changes in amino acid metabolism through glutaminolytic reprogramming. Glutaminolysis and levels of the key enzyme glutaminase are increased in TGFβ1-stimulated fibroblasts[71]. This leads to enhanced conversion of glutamine to glutamate, which confers resistance to apoptosis[72] and promotes the stabilization of collagen[71] via mTOR signalling. In vivo, the inhibition of glutaminase 1 ameliorates bleomycin- and TGFβ1-induced pulmonary fibrosis[73]. Changes in fatty acid oxidation have also been linked to fibrogenesis. Intracellular fatty acid oxidation is downregulated in tubulointerstitial fibrosis in mice and humans, and its restoration protects against fibrosis[74]. It has been reported that glycolysis is upregulated to compensate for reduced fatty acid oxidation during kidney injury, which could result in enhanced progression to fibrosis[70] (Fig. 3).

Key metabolic pathways such as increased glycolysis, upregulation of glutaminolysis and enhanced fatty acid oxidation are emerging as important drivers of fibroblast activation. No drugs that target these metabolic pathways have yet reached the clinic as antifibrotic therapies. However, drugs with known safety profiles that target metabolic pathways have been approved or are in clinical trials for the treatment of cancer, fuelling hope that in the future they could also be used to treat fibrotic diseases[75].

Macrophage-mediated regulation of fibrosis

Inflammatory monocytes and tissue-resident macrophages are key regulators of tissue fibrosis, playing important roles in the initiation, maintenance and resolution of tissue injury[76–78]. Furthermore, monocytes and macrophages can undergo remarkable functional plasticity, displaying diverse phenotypes during wound healing that depend on multiple cues including the environmental niche[79,80] and the temporal stage of tissue injury and repair[81,82]. Tissue macrophages are also

important producers of T cell- and fibroblast-recruiting chemokines, which orchestrate the development of the fibrotic niche[83].

Functional heterogeneity of monocytes and macrophages

Several studies have identified subpopulations of monocytes that can regulate fibrosis and tissue remodelling[22,79,84–87]. For example, a population of atypical monocytes characterized by carcinoembryonic antigen-related adhesion molecule-1 (CEACAM1$^+$ MSR1$^+$LY6C$^-$F4/80$^-$MAC1$^+$ monocytes)[84], which have been termed segregated-nucleus-containing atypical monocytes (SatMs) and share granulocyte characteristics, have a key role in lung fibrogenesis. SatMs are regulated by CCAAT/enhancer binding protein β (C/EBPβ), and *Cebpb* deficiency leads to a complete lack of SatMs. Bleomycin-induced fibrosis, but not inflammation, was inhibited in chimaeric mice with *Cebpb*$^{-/-}$ haematopoietic cells, and adoptive transfer of SatMs into *Cebpb*$^{-/-}$ mice resulted in fibrosis. Notably, SatMs are derived from Ly6C$^-$FcεRI$^+$ granulocyte/macrophage progenitors, but not from macrophage/dendritic cell progenitors. Single-cell RNA-seq approaches were used to investigate macrophage heterogeneity and function in the context of lung fibrosis. Although the main tissue-resident macrophage populations have been intensively studied, much less is known about the role of interstitial macrophages in fibrosis. Two independent subpopulations of interstitial macrophages that are conserved across lung, fat, heart, and dermis have been identified: LYVE1^{lo}MHCII^{hi}CX3CR1^{hi} (LYVE1^{lo}MHCII^{hi}) and LYVE1^{hi}MHCII^{lo}CX3CR1^{lo} (LYVE1^{hi}MHCII^{lo}) monocyte-derived interstitial macrophages. In a mouse model of inducible macrophage depletion (*Slco2b1*$^{flox/DTR}$), the absence of LYVE1^{hi}MHCII^{lo} interstitial macrophages exacerbates experimental lung fibrosis, thereby showing that two independent populations of interstitial macrophages coexist across tissues with conserved niche-dependent functional programs[79]. In addition, a pathological subgroup of transitional macrophages is required for the fibrotic response to injury in bleomycin-induced lung fibrosis. A computational approach that allows scRNA-seq data to be annotated by reference to bulk transcriptomes (SingleR) enabled macrophage subclustering and uncovered a disease-associated subpopulation with a transitional gene expression profile that is intermediate between monocyte-derived and alveolar macrophages. These CX3CR1$^+$SIGLECF$^+$ transitional macrophages localized to the fibrotic niche and were pro-fibrotic in vivo. This appears to be relevant to human disease, because human orthologues of genes expressed by these transitional macrophages were upregulated in samples from patients with IPF[86].

Research into the regulatory roles of monocytes and macrophages during tissue injury and repair has largely focused on blood-derived monocytes and macrophages. However, there is emerging evidence that resident cavity macrophages are also key contributors to fibrosis and tissue remodelling. For example, a reservoir of mature F4/80^{hi}GATA6$^+$ peritoneal cavity macrophages rapidly invades the liver via direct (avascular) recruitment across the mesothelium in response to sterile liver injury[88]. These recruited macrophages dismantle necrotic cell nuclei, releasing DNA and forming a cover across the site of injury[88]. Similarly, following myocardial infarction in mice, GATA6$^+$ macrophages in mouse pericardial fluid invade the epicardium and lose GATA6 expression but maintain antifibrotic properties, and loss of this macrophage population enhances interstitial fibrosis after an ischaemic injury. GATA6$^+$ macrophages are also found in human pericardial fluid, suggesting that this immune cardioprotective role for the pericardial tissue compartment may be relevant in human disease[89].

Macrophage and fibroblast cross-talk

Irrespective of how monocytes and macrophages are recruited into areas of tissue injury, pro-fibrotic macrophages commonly coordinate scar formation through a range of interactions with fibroblasts[90], which are the main cellular source of pathological ECM deposition during fibrosis[22,37,55,91–93]. For example, macrophage-derived amphiregulin has recently been shown to induce the differentiation of mesenchymal stromal cells into myofibroblasts via integrin-αV-mediated activation of TGFβ[94]. Previous work has shown that proximity is crucial to allow cross-talk between macrophages and contractile fibroblasts[37,92,93]; however, until recently it remained unclear how proximity between these two cell types is established. In an elegant study, contracting fibroblasts were shown to generate deformation fields in fibrillar collagen matrix that provided far-reaching physical cues to macrophages[95]. Within the collagen deformation fields created by fibroblasts or actuated microneedles, macrophages migrated towards the source of the force from distances of several hundred micrometres, and the presence of a dynamic force source within the matrix was required to initiate and direct macrophage migration. Notably, and counter to traditional views on how macrophages migrate within fibrotic tissues, the authors proposed that macrophages mechanosense the velocity of local displacements of their substrate, allowing contractile fibroblasts to attract macrophages over distances that exceed the range of chemotactic gradients[95].

Integrin-mediated activation of TGFβ

Secreted TGFβ is a major pro-fibrogenic cytokine, and therefore potentially represents an attractive antifibrotic target. Sustained systemic inhibition of TGFβ1, however, has undesired effects including cardiac valve problems, and TGFβ1-knockout mice develop systemic autoimmunity[96]. This is relevant to all mucosal surfaces, especially the intestine, where TGFβ1 activity is believed to control tissue homeostasis[97,98]. In addition, pan-TGFβ1 blockade has been found to induce carcinogenesis, perhaps owing to the role of TGFβ1 as an anti-proliferative mediator for most epithelial cell types. Some clinical trials using antibody-based pan-TGFβ blockade (for example, Fresolimumab) or TGFβ1 blockade (for example, Metelimumab) were terminated because of dose-limiting adverse events. Thus, strategies to avoid these deleterious effects could involve choosing the correct magnitude or duration of inhibition, co-administering anti-inflammatory therapies, or inhibiting TGFβ1 at specific sites in the tissue by blocking integrins and other mediators that locally activate latent TGFβ1.

The pericellular fibrotic matrix is a remarkably dynamic environment that exerts profound influences on cell behaviour, and many of the key cell–cell and cell–matrix interactions that regulate fibrosis are mediated by members of the integrin family (noncovalent α–β heterodimers with18 different α-subunits and 8 β-subunits, resulting in 24 known members in humans)[99]. Importantly, integrins can mediate the translation of spatially fixed extracellular signals into a wide variety of changes in cell behaviour, including alterations in cell adhesion, migration, proliferation, differentiation and apoptosis[99,100]. Of key relevance to fibrosis, integrins can also potentiate signals from soluble pro-fibrogenic growth factors such as TGFβ1. Nearly all TGFβ1 is secreted and bound to the ECM in a latent form, and therefore the majority of the regulation of TGFβ function during fibrosis depends on site-specific regulation of TGFβ activation, rather than its synthesis or secretion[101].

The most intensively studied mechanism for activation of TGFβ1 is the interaction of the TGFβ1 latent complex with the αv-containing subset of integrins. Specifically, the integrins αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8 have all been shown to bind to an N-terminal fragment of the TGFβ1 gene product called the latency associated peptide (LAP), which forms a noncovalent complex with the active cytokine, preventing latent TGFβ from binding to its cognate receptors and inducing biological effects[102–104]. When a mechanical force is applied to the latent complex by contraction of αvβ6 integrin-expressing cells, the resultant conformational change leads to the release of active TGFβ1[105–107]. Notably, a recent study has shown that in contrast to this αvβ6-mediated mechanism of TGFβ activation, αvβ8-dependent activation of TGFβ can occur independently of actin-cytoskeletal force and does not require

the release of mature TGFβ[108], further highlighting the complexity of αv integrin-mediated TGFβ activation.

There are now abundant preclinical data across a range of fibrotic disease models demonstrating critical regulatory roles for αv-containing integrins expressed on various different cell lineages. Mice lacking the αvβ6 integrin are protected in mouse models of lung, kidney and biliary fibrosis[103,109–111]. This protection is secondary to local inhibition of TGFβ, and antibody-mediated inhibition of αvβ6-mediated TGFβ1 activation decreased lung fibrosis in preclinical models[112,113]. TGFβ1 activation by the αvβ8 integrin represents a further potential therapeutic target[114,115]. Conditional depletion of αvβ8 integrin in lung fibroblasts inhibited experimental airway fibrosis[115], and, in mice genetically engineered to replace the mouse β8 subunit with its human orthologue, a blocking antibody against human αvβ8 blocked TGFβ1 activation and protected against allergic airway inflammation and remodelling induced by cigarette smoke[116]. Furthermore, depletion of the αv integrin subunit on mesenchymal cells also inhibited fibrosis in models of liver, lung and kidney fibrosis[117]. The depletion of αv integrins on hepatic myofibroblasts in *Pdgfrb*-Cre mice protected the mice against hepatic fibrosis, whereas global loss of β3, β5 or β6 integrins, or conditional loss of β8 integrins in myofibroblasts, did not; this highlights the context dependency of the regulation of fibrosis in different organs by the various αv-containing integrins. Pharmacological blockade of αv-containing integrins by a small-molecule inhibitor (CWHM 12) attenuated both liver and lung fibrosis, even when fibrosis was already established[117]. Tissue fibroblasts can express four αv-containing integrins—αvβ1, αvβ3, αvβ5 and αvβ8. Selective small-molecule inhibitors of αvβ1 have been used to investigate the role of this integrin, with studies demonstrating that αvβ1 blockade has an antifibrotic effect in models of lung and liver fibrosis[102].

Given the abundance of preclinical data, this remains a very active area of research and development in the fibrosis field, with multiple small-molecule and antibody-based approaches undergoing assessment in clinical trials, including inhibitors designed to selectively target multiple αv-containing integrins simultaneously. This includes phase 2 trials of inhibitors of αvβ6 (NCT01371305), αvβ1 and αvβ6 (NCT04072315), and αvβ1, αvβ3 and αvβ6 (NCT03949530), all in pulmonary fibrosis (Supplementary Table 2). Patient safety will be an important consideration in these trials, as Biogen recently terminated their trial of a selective anti-αvβ6 antibody in patients with IPF owing to safety concerns.

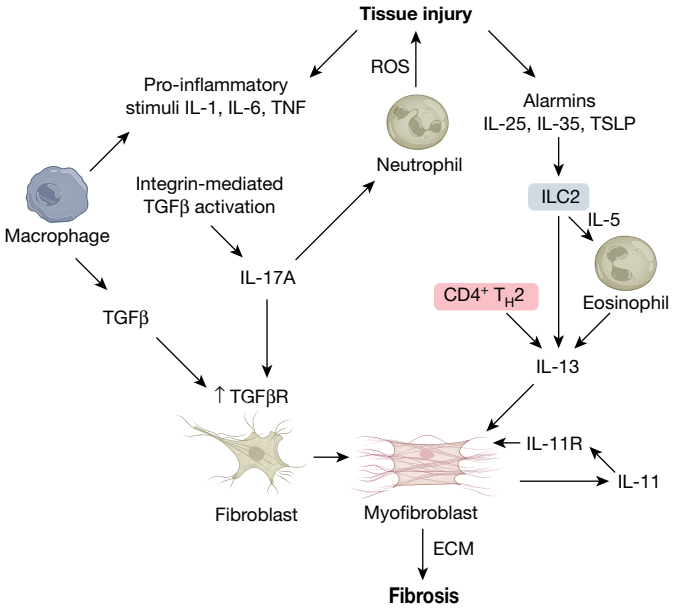

Fig. 4 | Divergent cytokine pathways drive fibrosis. The innate acute-phase pro-inflammatory cytokines IL-1, IL-6, and TNF, together with TGFβ, which are produced by macrophages, tissue fibroblasts, and other local cell populations, promote the development of IL-17-secreting cells. IL-17A potentiates neutrophil responses that contribute to tissue injury through the production of reactive oxygen species (ROS), while increasing the expression of TGFβ receptors on fibroblasts and thereby facilitating the production of ECM in response to TGFβ. TGFβ is a key driver of fibrosis that is produced and activated locally through integrin-mediated mechanisms. A second and distinct cytokine-mediated pathway that can promote fibrosis independently of TGFβ is the type 2 cytokine axis. Here, the alarmin cytokines IL-25, IL-35, and thymic stromal lymphopoietin (TSLP), secreted by epithelial cells and other damaged tissues, drive the expansion and activation of type 2 innate lymphoid cells (ILC2s) that secrete large amounts of IL-5 and IL-13. IL-5 in turn drives the recruitment and activation of local tissue eosinophils, which provide an additional source of type 2 cytokines and other pro-fibrotic mediators. IL-13, which is derived from eosinophils, $CD4^+$ type 2 T helper (T_H2) cells, and ILC2s, exhibits potent pro-fibrotic activity that is independent of TGFβ. Finally, the cytokine IL-11, which is produced by activated myofibroblasts, stimulates ECM production by myofibroblasts in response to multiple pro-fibrotic mediators, including TGFβ and type 2 cytokines.

Cytokine-mediated regulation of fibrosis

Other than TGFβ, several additional cytokines that are secreted from multiple cellular sources have been identified as triggers of fibrosis[118]. The pro-inflammatory cytokine interleukin 17A (IL-17A) can induce fibrosis in different organ systems, including the lung, liver, kidney, heart and skin[119–124]. In a study of bleomycin-induced pulmonary fibrosis, IL-17A produced by γδ and $CD4^+$ T cells induced lung inflammation, neutrophil recruitment, and production of TGFβ[120]. Neutrophils and mast cells are also important sources of IL-17A[125]. Experiments with mice lacking IL-17A or its receptor IL-17RA, as well as therapeutic studies using IL-17A-neutralizing antibodies, confirmed that IL-17A signalling is involved in fibrosis in multiple tissues[120–122,126–128]. As well as promoting TGFβ production[126], IL-17A increases and stabilizes the expression of TGFβRII on fibroblasts, thereby enhancing their sensitivity to TGFβ[129]. The T_H17-associated cytokine IL-22 similarly enhances TGFβ signalling in fibroblasts[125]. TGFβ in turn induces the expression of IL-17A when produced concurrently with the pro-inflammatory cytokines IL-1, IL-6, or TNF[120,130,131], suggesting that a feed-forward mechanism that involves acute-phase cytokines, IL-17A, and TGFβ is responsible for the development of fibrosis following acute injury[119,120,132] (Fig. 4); IL-17A exhibits similar activity in animal studies and human cells[133,134].

Caspase 1, the NOD, LRR and pyrin domain-containing (NLRP) 3 inflammasome, and NFκB were identified as important upstream activators of the IL-17A–TGFβ axis[131]. The mechanisms responsible for the sustained activation of NFκB and NLRP3 inflammasome signalling remain unclear, although commensal microorganism stimulation of Toll-like receptors on myeloid cells and tissue fibroblasts has been hypothesized to be an important activating mechanism, with the resulting pro-inflammatory cytokine and chemokine production exacerbating inflammation and the progression of fibrosis[135,136]. Of note, stimulation of TLR4 or NFκB in hepatic stellate cells enhances TGFβ signalling by directly downregulating the TGFβ pseudoreceptor BMP and the activin membrane-bound inhibitor Bambi[136]. A related study showed that sustained activation of the NLRP3 inflammasome is associated with increased chemokine expression, recruitment of neutrophils and macrophages, and persistent production of IL-17A and TNF[131]. Thus, activation of the pro-fibrotic TGFβ signalling pathway is driven by several collaborating mechanisms, with IL-17A having a prominent role.

Whereas the TGFβ superfamily of ligands are well-known drivers of fibrosis[137], the type 2-associated cytokines IL-4 and IL-13 have also emerged as distinct but important inducers of fibrosis. Here, the fibrotic response is associated with predominant infiltration of

eosinophils and M2-like macrophages, rather than the neutrophil and M1-like monocyte/macrophage phenotype that characterizes the IL-1–IL-17A–TGFβ axis[138]. Also, instead of acute-phase cytokines serving as co-inducers, the alarmin cytokines thymic stromal lymphopoietin, IL-25, and IL-33 function as key initiators of type 2-dependent fibrosis by triggering the production of IL-4 and IL-13 in innate lymphoid cells, T cells, eosinophils, and other type 2-associated leukocytes[139–142]. Although IL-13 can induce and activate TGFβ in macrophages[143], it may promote fibrosis independently of TGFβ[144] in part by directly targeting stromal and parenchymal cells, including epithelial populations and collagen-producing myofibroblasts[145]. Mice deficient in IL-13, IL-4R, or IL-13Rβ1, as well as animals treated with neutralizing antibodies to IL-13 or IL-4R, show reduced fibrosis after many types of tissue injury[146–150], confirming that type 2 cytokine signalling is critical in the progression of fibrosis (Fig. 4).

The mechanisms that dictate whether the IL-1–IL-17A–TGFβ axis or the type 2 cytokine response dominates as the key driver of fibrosis remain unclear, although the type of cellular damage or duration of the injury are likely to be important. For example, studies with the commonly used 'single hit' bleomycin model of pulmonary fibrosis revealed a prominent role for the IL-1–IL-17A–TGFβ axis but little to no contribution for type 2 cytokines, despite substantial upregulation of IL-4 and IL-13 in the lungs[120]. Nevertheless, a modified version of this model in which bleomycin was injected intradermally rather than intratracheally over several weeks uncovered a substantial role for IL-4R signalling in the development of pulmonary fibrosis[151]. Different stimuli or types of injuries that lead to the preferential production of alarmin cytokines versus the activation of NF-κB and inflammasome signalling are also likely to have key roles. For example, integrin receptors that interact with the ECM preferentially activate TGFβ signalling and the production of IL-17A while antagonizing the production of type 2 cytokines[152]. Consistent with these observations, several studies have revealed substantial cross-regulation between IL-17A and IL-13[120,153], with marked upregulation of the opposing pathway when one mechanism was targeted therapeutically[146,152,154,155]. Consequently, a successful antifibrotic strategy may need to target the dominant mechanism or reduce both pathways simultaneously. IL-11, a member of the IL-6/gp130 cytokine family, may be a promising target, as it was recently shown to integrate pro-fibrotic signals emanating from both pathways[156–158].

Not surprisingly, given the robust preclinical data, inhibitors of IL-13 alone (NCT01266135, NCT00987545, NCT00581997, NCT01872689 and NCT01629667) or a combination of IL-4 and IL-13 inhibitors (NCT02921971 and NCT01529853) have been tested in phase 2 trials for pulmonary fibrosis, skin keloids, and systemic sclerosis. Although the results so far have been mostly negative or mixed, Romilkimab (SAR156597), a bi-specific antibody against IL-4 and IL-13, did have a significant effect on modified Rodnan skin score in a 24-week study of diffuse cutaneous systemic sclerosis (NCT02921971). Adalimumab, an antibody against TNF, is also being tested in Dupuytren's disease, a complex fibroproliferative disease of the hand (NCT03180957). Additional cytokine, chemokine or growth factor inhibitors in development for fibrosis in phase II or III trials are inhibitors of CCR2 and CCR5 (NCT02217475, NCT03028740, NCT03059446 and NCT02330549) for liver fibrosis and NASH, an inhibitor of IL-1 (NCT01538719) for systemic sclerosis, an inhibitor of IL-6 (NCT02453256) for scleroderma, an inhibitor of CCL2 (NCT00786201) for pulmonary fibrosis, and follistatin (an activin antagonist) for Beckers muscular dystrophy (NCT 01519349) (Supplementary Table 2).

Contribution of the microbiome to fibrosis

Most human-associated microorganisms are found in the gut, and during homeostasis these microbial populations are essential for maintaining gut health. However, when the balance between healthy and pathogenic microorganisms shifts towards pathogenic subsets, disease can ensue. Inflammatory bowel disease (Crohn's disease or ulcerative colitis) represents a prototypical pathology in which dysbiosis is thought to be a key driver of disease pathogenesis, with evidence that there is a strong link between the microbiota and the development of fibrosis. For example, patients with Crohn's disease carrying variants of the *NOD2* gene, which encodes an intracellular pattern recognition receptor, are at increased risk of stricture formation, which is the major manifestation of intestinal fibrosis[159]. Furthermore, serologic antimicrobial antibodies are common in patients with Crohn's disease and are associated with and predictive of intestinal strictures[160,161], and almost all mouse models of intestinal fibrosis are influenced by the microbiota[162]. For instance, global deletion of the bacterial signalling adaptor molecule MyD88[163] reduced intestinal fibrosis in a mouse model of *Salmonella*-induced colitis[164]. Microbiota-driven intestinal fibrosis may be mediated by induction of the IL-33 receptor ST2 on epithelial cells[165] or by the pro-fibrotic action of TL1A[166]. On a cellular level, TLR2 or TLR4 ligands induce secretion of cytokines and chemokines from cultured intestinal myofibroblasts[167]. Interestingly, although human intestinal mesenchymal cells express multiple TLRs and NLRs, one study found that a pro-fibrogenic phenotype was triggered exclusively by flagellin, a broad activator of innate and adaptive immunity and a TLR5 ligand. This occurred in a TGFβ1-independent manner and via post-transcriptional regulation[168]. The role of myofibroblasts in directly sensing pathogen-associated molecular patterns in intestinal fibrosis has been confirmed in vivo, as selective deletion of MyD88 in cells expressing α-smooth muscle actin (α-SMA) ameliorated intestinal fibrosis[168].

Dysbiosis in the gut also influences liver fibrosis. Translocation of bacteria and their products across the intestinal barrier owing to intestinal barrier disruption is common in patients with chronic liver disease. Increased levels of the microorganism-derived ligand lipopolysaccaride (LPS) in the portal vein or translocation of whole bacteria or their products to the liver activates inflammation that leads to fibrosis[169]. Blocking TLR4 signalling in mice or reducing hepatic exposure to intestinal microorganisms by reducing microbial load with antibiotics ameliorates experimental liver fibrosis[136]. HSCs express all known human TLRs and respond to TLR4 ligands[136,170], which also downregulate a TGFβ1 decoy receptor and thereby sensitize HSCs to the action of TGFβ1[136]. A comparable mechanism has been described in pancreatic fibrosis in rats[171]. TLR4 signalling includes an additional signalling adaptor, called TRIF (also known as TICAM1). Deletion of TRIF in a mouse model of diet-induced NASH reduced hepatic steatosis but increased hepatic fibrosis, and *Trif*$^{-/-}$ HSCs expressed higher levels of CXCL1 and C-C motif chemokine ligands in response to LPS, highlighting a potential mechanism for this unexpected effect[172]. Conversely, distinct gut microbiota may be hepatoprotective in liver fibrosis. ECM deposition in the liver was higher in germ-free mice than in conventionally housed mice[173], and MyD88- and TRIF-deficient mice showed the same effect. In the kidney, pericytes (a myofibroblast precursor) activate a TLR2–TLR4–MyD88-dependent pro-inflammatory program in response to tissue injury[174]. The downstream kinase IRAK4 controls the conversion of pericytes into myofibroblasts in vitro, and pharmacological inhibition of MyD88 signalling with an IRAK4 inhibitor reduced fibrosis by attenuating tissue injury in vivo[174]. Global TLR4 knockout[175] and a small-molecule inhibitor of MyD88 ameliorate renal fibrosis in mice[176]. In human systemic sclerosis, TLR4 and its co-receptors lymphocyte antigen 96 (MD2) and CD14 are overexpressed in lesional skin and chronic dermal LPS exposure leads to overexpression of TGFβ signature genes[177].

Several TLRs are promiscuous and can also sense damage-associated molecular patterns, lipids or ECM. For example, in lung fibrosis TLR4 and the glycosaminoglycan hyaluronan are important for type 2 alveolar epithelial cell renewal, which limits lung injury and fibrosis[178]. Notably, TLR4 was protective in the lung, as opposed to its pathogenic effect in gut and liver fibrosis[136,167]. Nasal polyposis is a disease characterized

by remodelling of the sinonasal mucosa. Short single-stranded DNA molecules (CpG oligonucleotides) can activate fibroblasts derived from patients with nasal polyposis via TLR9 stimulation, providing an additional example whereby multiple pattern recognition receptors, activated by distinct ligands, can contribute to aberrant wound healing and fibrosis.

The pathophysiological relevance of TLR4 in inflammation has led to clinical trials of a TLR4 inhibitor for treating rheumatoid arthritis (NCT03241108). Furthermore, pentraxin 2 (PTX2), also known as serum amyloid protein 2, has demonstrated anti-inflammatory and antifibrotic properties in multiple preclinical fibrosis models[179,180] and recombinant PTX2 (PRM-151) has entered phase II trials for pulmonary fibrosis and myelofibrosis (NCT02550873; NCT01981850; Supplementary Table 2).

Future directions

Fibrosis is a major global healthcare burden. Consequently, the discovery of key therapeutic targets with high relevance to human fibrotic disease and the subsequent development of effective antifibrotic therapies directed against these targets continues to be a research priority. The only two drugs that have been approved in several countries so far for the treatment of a fibrotic disease are nintedanib and pirfenidone, both for patients with IPF[181]. Nintedanib also recently received approvals for the treatment of systemic sclerosis-associated interstitial lung disease and progressive fibrosing interstitial lung diseases. Thus, drug development in this important field remains limited, has been restricted to only one organ system, and continues to progress slowly. Single-cell genomics methodologies have already yielded new discoveries that would previously have been unattainable. This field continues to evolve rapidly, and emerging technologies are now able to measure multiple omic readouts (genomes, epigenomes, transcriptomes and proteomes) in single cells[182–184]. Spatially resolved molecular profiling is expanding our understanding of how these populations interact in situ[185–187]. The convergence and integration of these multi-modal single-cell technologies[24,188], alongside global initiatives such as the Human Cell Atlas[189], represent an extraordinary opportunity to decode the cellular and molecular mechanisms of fibrosis at unprecedented resolution, which should in turn help to drive a new era of precision medicine in the treatment of fibrotic disease. Novel therapeutics developed for one fibrotic disorder may be applicable to a wide range of fibrotic diseases because of the shared pathways across organs that are uncovered by this work. Drug repositioning efforts may also be assisted by these studies[190].

Despite impressive progress over the past few years in our understanding of the pathogenesis of fibrosis, multiple challenges need to be overcome to translate this information into effective antifibrotic therapies (Fig. 5). Prognostic animal models and ex vivo primary human tissue culture systems need to be developed that allow better translation of novel mechanisms from the bench to the bedside. Patient heterogeneity, together with the fact that fibrosis progression is typically slow, makes the selection of patients for clinical trials difficult[26]. Hence, accurate and validated predictors of fibrotic disease progression are needed to stratify patients into high-risk populations before their inclusion in trials. In fact, groups of human fibrotic diseases may be subdivided on a mechanistic basis using analysis of tissue samples. Subsets of patients could then be targeted with personalized antifibrotic therapies. Work in this area should be a research priority. At present, trial end-points are highly variable and often lack the sensitivity needed to predict favourable responses over a short period of time, which necessitates the inclusion of large numbers of patients in clinical trials. Consequently, end-points for fibrosis clinical trials continue to evolve and may require a more global approach involving scientists, industry leaders, patients and regulatory partners, as shown for liver fibrosis and intestinal fibrosis[191,192]. Ideally, non-invasive end-points

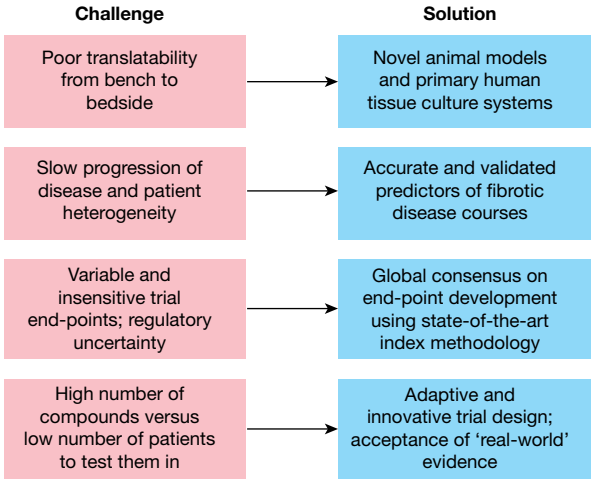
Challenge
Solution
Poor translatability from bench to bedside
Novel animal models and primary human tissue culture systems
Slow progression of disease and patient heterogeneity
Accurate and validated predictors of fibrotic disease courses
Variable and insensitive trial end-points; regulatory uncertainty
Global consensus on end-point development using state-of-the-art index methodology
High number of compounds versus low number of patients to test them in
Adaptive and innovative trial design; acceptance of 'real-world' evidence

Fig. 5 | Challenges and solutions in the translation of antifibrotic mechanisms into drugs. The red boxes on the left describe some of the major challenges in the development of antifibrotic drugs that have been described in this Review, with the arrows pointing to potential solutions in the blue boxes.

that better correlate with clinically meaningful outcomes are needed. Recent research in the field has been fueled by the discovery of robust biomarkers and cutting-edge imaging modalities such as PET imaging of collagen and molecular imaging of fibrosis[193,194], which allows fast, non-invasive, and whole-organ-quantitative and longitudinal readouts of drug efficacy in antifibrotic clinical trials. Furthermore, combining molecular imaging of fibrosis[193] with cutting-edge omic approaches, such as single-cell genomics[182–184], could markedly improve patient diagnostics, staging, prognostication, stratification and cohort enrichment, which would in turn optimize clinical trial design and maximize the number of trials that could be run quickly and efficiently[185–187]. Innovative approaches to trial design are being developed that allow the incorporation of adaptive strategies and the use of 'bucket' trials that include patients with different types of fibrosis, as well as the inclusion of 'real-world' evidence into the regulatory approval process.

Similar to the major advances seen in cancer therapy and the successful treatment of HIV and viral hepatitis, it is likely that we will see increasing numbers of clinical trials testing combinations of drugs to treat fibrosis, as fibrosis is increasingly recognized as a highly complex disorder, with multiple mechanisms collaborating to drive disease progression. These antifibrotic drug cocktails will probably target a variety of orthogonal mechanisms, including a range of receptors, signalling pathways, and cell types that have been shown to function as core drivers of fibrosis in multiple disease states. These multifaceted approaches should pave the way towards the delivery of effective antifibrotic therapies in the future.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2938-9.

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Additional information

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